Synthesis and Cytotoxicity of 2-Methyl-4,9-dihydro-1-substituted-1*H*-imidazo[4,5-*g*]quinoxaline-4,9-diones and 2,3-Disubstituted-5,10-pyrazino[2,3-*g*]quinoxalinediones

Hee-Won Yoo,[†,‡] Myung-Eun Suh,[†] and Sang Woo Park*[,‡]

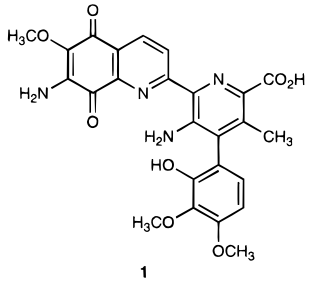
Division of Medicinal Chemistry, College of Pharmacy, Ewha Womans University, Seoul 120-750, Korea, and Medicinal Chemistry Research Center, Korea Institute of Science and Technology, P.O. Box 131, Cheongryang, Seoul 130-650, Korea

Received October 14, 1997

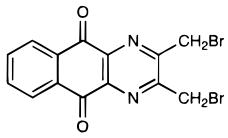
Series of 2-methyl-4,9-dihydro-1*H*-imidazo[4,5-*g*]quinoxaline-4,9-diones and 5,10-pyrazino[2,3-*g*]quinoxalinediones have been synthesized from 6,7-dichloro-5,8-quinoxalinedione for developing new anticancer drugs. Intercalation complex of 2-methyl-4,9-dihydro-1-methyl-1*H*-imidazo-[4,5-*g*]quinoxaline-4,9-dione with GC/GC base pairs by a computer graphics-aided method was parallel to the axis of the helix. The interaction energies of synthetic compounds were low. 2-Methyl-4,9-dihydro-1-(4-bromophenyl)-1*H*-imidazo[4,5-*g*]quinoxaline-4,9-dione, which has the lowest interaction energy of the test compounds, was identified as being more cytotoxic against human gastric adenocarcinoma cells (MKN 45) than adriamycin and *cis*-platin in vitro using the MTT assay. The IC_{50} value of this compound was 1.30 μM, whereas those of adriamycin and *cis*-platin were 3.13 and 86.5 μM, respectively. The cytotoxicity of 2,3-diethyl-5,10-pyrazino-[2,3-*g*]quinoxalinedione was comparable to that of adriamycin in the in vitro assay. However these compounds showed loss of activity on human lung adenocarcinoma cells (PC 14) and human colon adenocarcinoma cells (colon 205). These results suggest that 2-methyl-4,9-dihydro-1-(4-bromophenyl)-1*H*-imidazo[4,5-*g*]quinoxaline-4,9-dione, with the lowest interaction energy, might be an excellent and selective antitumor agent against MKN 45.

Introduction

Streptonigrin (**1**), obtained from *Streptomyces floccu-lus*, was known as an antitumor agent by Rao and Cullen in 1959,[1] but application is limited because of its toxicity.[2,3] The 7-amino-6-methoxy-5,8-quinolinedi-one moiety in **1** is responsible for antitumor activity.[4,5] Studies on the activity of heterocyclic quinones contain-ing nitrogen such as quinolinedione showed that the number and position of nitrogens are considerably important for the cytotoxicity.[6] Thus the diazanaphtho-quinones were proved to be the most active compounds in comparison with naphthoquinone and quinolinedione. It was published that 5,8-quinoxalinediones that have one additional nitrogen in the nucleus different from quinolinedione showed antitumor activity.[7] Another structural requirement for the antitumor activity is the *p*-quinone moiety in the nonheterocyclic ring, whereas *o*-quinone gave decreased activity.[8,9] The electron-withdrawing groups at the 6 and 7 positions of quino-linediones also contributed to the activity,[10] and more annelated heterocyclic quinones were reported to in-crease the antitumor activity.[11] Giorgi-Renault pre-pared benzoquinoxalinediones and pyridoquinoxalined-iones, which are quinoxalinediones fused with benzene or pyridine, and examined their antitumor activities.[12] These tricyclic quinones exhibited cytotoxicity; espe-cially 2,3-bis(bromomethyl)-5,10-benzo[*g*]quinoxalined-ione (**2**) was highly active against sarcoma 180. This led us to think that replacement of the benzene ring of



1



2

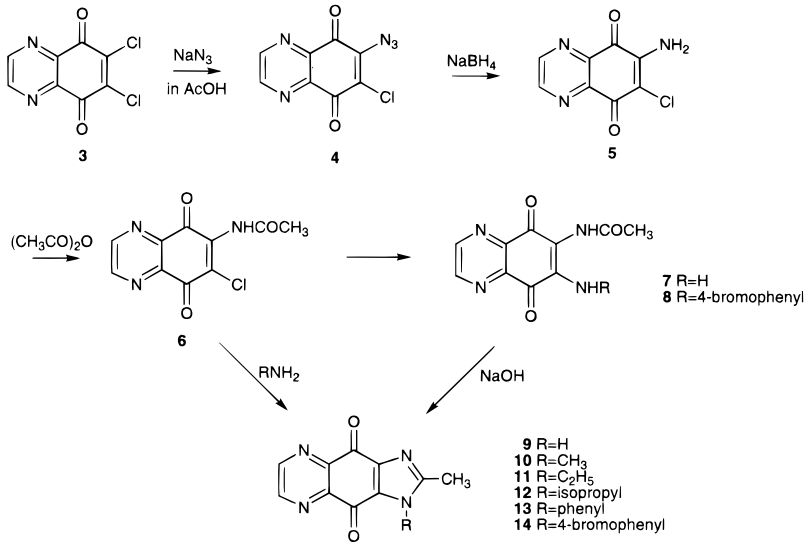
benzoquinoxalinedione with pyrazine or imidazole het-erocyclic rings might improve the activity. We report here the synthesis of chemical modifications on hetero-cycles like imidazoquinoxalinedione and pyrazinoqui-noxalinedione derivatives. In a previous paper[13] 6,7-dichloro-5,8-quinoxalinedione (**3**) was prepared in rela-tively good yield, while synthesis of imidazoquinoxa-linediones and pyrazinoquinoxalinediones had not yet been described. 6,7-Dichloro-5,8-quinoxalinedione was substituted with nucleophiles easily. Therefore we could obtain heterocyclic compounds with more nitro-gens from 6,7-dichloro-5,8-quinoxalinedione as a start-ing material.

One of the cytostatic action mechanisms of coplanar polycyclic compounds is their intercalation with human DNA. This caused enzymatic blockade and reading errors during the replication process.[14] If the com-pounds have three to four rings which are coplanar, they would give the optimal intercalation. The intercalation of a synthetic compound between two base pairs and their interaction energies were investigated by computer-aided molecular modeling. The synthetic compounds described here are coplanar annelated quinoxalinedione

* Corresponding author: Sang Woo Park. Phone: 82-2-958-5132. Fax: 82-2-958-5189.
[†] Ewha Womans University.
[‡] Korea Institute of Science and Technology.

10.1021/jm970695n CCC: $15.00
Published on Web 10/24/1998

Scheme 1



analogues and might interact with DNA. On the basis of this consideration, they might be expected to possess antitumor activity. In order to examine the effect of side chains, the alkyl and aryl groups were substituted on the nitrogen nucleus.

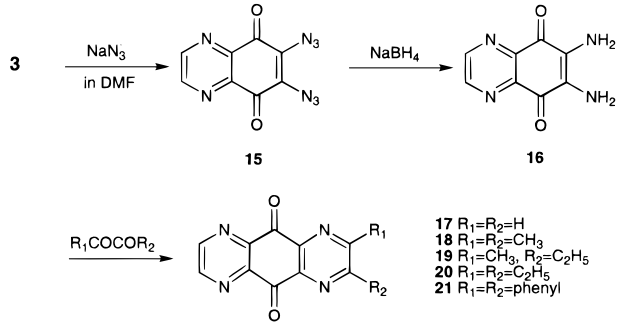
Results And Discussion

Synthetic Chemistry. 6-Azido-7-chloro-5,8-quinoxalinedione (**4**) was obtained by treating 6,7-dichloro-5,8-quinoxalinedione (**3**) with sodium azide in acetic acid and then was reduced by sodium borohydride to 6-amino-7-chloro-5,8-quinoxalinedione (**5**). When 6,7-dichloro-5,8-quinoxalinedione was treated with ammonia, it was reduced to 6,7-dichloro-5,8-dihydroxyquinoxaline. This was confirmed by acetylation of 6,7-dichloro-5,8-dihydroxyquinoxaline which gave 6,7-dichloro-5,8-diacetoxyquinoxaline identified from NMR and MS spectrometry. Acetylation of **5** produced 6-acetamido-7-chloro-5,8-quinoxalinedione (**6**). 6-Acetamido-7-amino-5,8-quinoxalinedione (**7**) was prepared by reaction of (acylamino)-quinone with sodium azide and reduction with sodium borohydride. 6-Acetamido-7-[(4-bromophenyl)amino]-5,8-quinoxalinedione (**8**) was obtained by reacting compound **6** with 4-bromoaniline in ethanol.

The conversion of the 6-acetamido-7-amino-5,8-quinoxalinedione (**7**) and 6-acetamido-7-[(4-bromophenyl)-amino]-5,8-quinoxalinedione (**8**) to the corresponding imidazoles (**9**, **14**) was readily performed under acidic or basic conditions by the method of Fries and Billig.[15] However 2-methyl-4,9-dihydro-1-alkyl-1*H*-imidazo[4,5-*g*]quinoxaline-4,9-diones (**10**−**12**) and 2-methyl-4,9-dihydro-1-phenyl-1*H*-imidazo[4,5-*g*]quinoxaline-4,9-dione (**13**) were prepared directly by reaction of 6-acetamido-7-chloro-5,8-quinoxalinedione (**6**) with alkylamine and phenylamine without catalyst (Scheme 1).

With respect to the pyrazinoquinoxalinedione derivatives, 6,7-diamino-5,8-quinoxalinedione (**16**) was synthesized by reduction of diazidoquinoxalinedione (**15**) with sodium borohydride which was produced by reacting 6,7-dichloro-5,8-quinoxalinedione (**3**) with sodium azide in dimethylformamide.[16] 6,7-Diamino-5,8-quinoxalinedione (**16**) was also obtained by deacetylation of 6-acetamido-7-amino-5,8-quinoxalinedione (**7**) under basic conditions.[17]

Scheme 2



Reaction of diaminoquinoxalinedione (**16**) with glyoxal in water[18,19] gave 5,10-pyrazino[2,3-*g*]quinoxalinedione (**17**). 2,3-Dialkyl-5,10-pyrazino[2,3-*g*]quinoxalinediones (**18**−**20**) were prepared by reaction of diaminoquinoxalinedione with 1,2-diketones in 50% aqueous acetic acid.[20] Acetic acid was necessary in this reaction. Attempts to prepare pyrazinoquinoxalinedione derivatives without acid[21] were unsuccessful. 2,3-Diphenyl-5,10-pyrazino[2,3-*g*]quinoxalinedione (**21**) was obtained by reaction of 6,7-diamino-5,8-quinoxalinedione (**16**) with benzil in ethanol using concentrated sulfuric acid as a catalyst (Scheme 2). Reaction of diaminoquinoxalinedione with 1,4-dibromo-2,3-butanedione did not give 2,3-bis(dibromomethyl)-5,10-pyrazino[2,3-*g*]quinoxalinedione but 2,3-dimethyl-5,10-pyrazino[2,3-*g*]quinoxalinedione (**18**). The bromomethylated quinone with, the great lability of bromine atoms, easily reacted with nucleophiles like water.[12] Dehalogenation occurred under this condition using 50% aqueous acetic acid as a solvent.

Molecular Modeling of Intercalation Complexes and Their Interaction Energies. Intercalation of compounds with human DNA is the insertion of the planar part of a molecule between two stacked base pairs. Hydrogen bonds make a major contribution to the stability of the complex. The primary and secondary structures of DNA in the complex remain unchanged. However the DNA tertiary structure in the intercalation complex is somewhat lengthened and unwound in comparison with the original structure.[22] Complete energy maps can be represented by molecular modeling, and it is possible to follow molecular changes visually

Table 1. Interaction Energies (ΔE) of DNA−Intercalator complexes

compd no.	intercalator MAXIMIN2 energy (kcal/mol)	DNA−intercalator complex energy (kcal/mol)	interaction energy (kcal/mol)
10	39.232	27.307	−11.925
11	37.212	29.187	−8.025
12	41.228	29.881	−11.347
14	39.222	24.574	−14.648
18	14.695	3.678	−11.017
19	6.478	−4.457	−10.929
20	15.300	0.836	−14.464

and to calculate defined interaction energies. This would allow us to expect their activities. Molecular spectroscopic methods have contributed considerably to the molecular resolution of an intercalation complex. The molecule should have three to four planar rings (minimum surface area of 28 Å^2), and the intercalation complex should be parallel to the axis of the helix for an ideal intercalation.[23] Normally the compound which has a lower interaction energy is more cytotoxic than the compound which has a higher interaction energy. This is true only when binding energy is more important than other factors such as cellular uptake and bioactivation.

At first, a theoretical study from the intercalation complex of synthetic compounds with cytidylyl(3′−5′)-guanosine was examined using molecular mechanics method, here especially MAXIMIN2 force field in the Sybyl software program (hardware: Indy, 6.2 version, Tripos Co., St. Louis, MO). The structures of compounds were constructed with the option *Crysin* in Sybyl, and the low-energy conformers of compounds were determined by the standard Sybyl energy minimizer, MAXIMIN2, which was used under default conditions. The cocrystal structure of the synthetic compound with cytidylyl(3′−5′)guanosine was built on a molecular graphics system using the geometry data of a GC base pair model taken from the published structure,[24] and then geometric optimization of the complex was carried out. The partial charges used in the calculations were generated by the Gasteiger−Hückel method using the Tripos force field in the Sybyl molecular modeling package. Gradient norm was 0.01 kcal/mol, and 400 iterations were performed for the optimization. Each unit of the structure consists of one synthetic compound and two dinucleoside monophosphate CpG molecules. The solvent and counterions were not used in the models. The interaction energies of DNA−intercalator complexes are summarized in Table 1.

A modeling study of the 2:1 complex between cytidylyl(3′−5′)guanosine and 2-methyl-4,9-dihydro-1-methyl-1*H*-imidazo[4,5-*g*]quinoxaline-4,9-dione (**10**) has led to a general DNA binding model in which the compound chromophore intercalates between base pairs (Figure 1). As expected the DNA intercalation complex is parallel to the axis of the helix, and the synthetic compound is perpendicular to the line joining the base pairs. This intercalator, which is the coplanar compound with three rings, especially shows the advantageous arrangement, and its interaction energy is −11.925 kcal/mol. We could see only hydrogen atoms out of plane. It proves that the coplanar imidazoquinoxalinediones and pyrazinoquinoxalinediones would make good intercalation

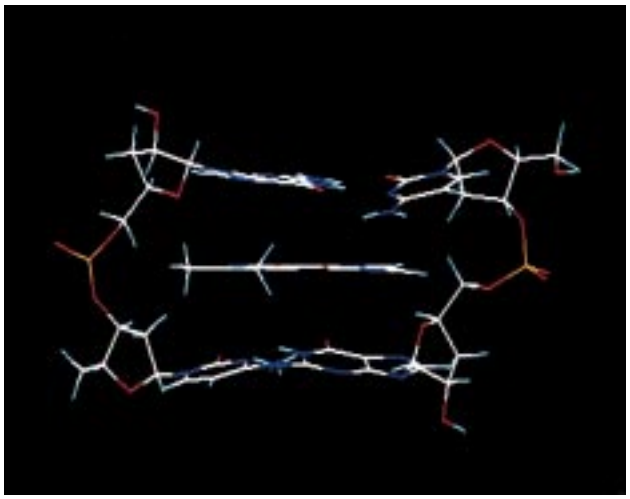


Figure 1. Molecular structure of 2-methyl-4,9-dihydro-1-methyl-1*H*-imidazo[4,5-*g*]quinoxaline-4,9-dione (**10**) with cytidylyl(3′−5′)guanosine.

complexes. Interestingly the interaction energy of 2-methyl-4,9-dihydro-1-(4-bromophenyl)-1*H*-imidazo-[4,5-*g*]quinoxaline-4,9-dione (**14**) substituted with a 4-bromophenyl at the 1 position is −14.648 kcal/mol which is the lowest energy examined. 2,3-Diethyl-5,10-pyrazino[2,3-*g*]quinoxalinedione (**20**), substituted with diethyl groups at the 2 and 3 positions, also has an interaction energy of −14.464 kcal/mol. This suggests that **14** and **20** with bulky side chains would interact with DNA more strongly than the others. Thus they would form stable intercalation complexes and might have high activity.

The compound **10**, as a representative of synthetic compounds, has been tested to see if it interacts with DNA, using ultraviolet spectrophotometric means and an unwinding test.[25] The UV spectroscopic properties of **10** in the presence and absence of DNA were studied by conventional optical spectroscopy. Characterization of this mixture was done by comparing the ultraviolet absorption spectrum of the complex with the spectrum of the sample. The DNA-bound **10** showed hyperchromism and a red shift in the absorption band. These spectral changes suggest binding of the compound on the base pairs (data not shown). In general the ability of a drug to unwind circular supercoiled DNA molecules is also considered to be one of the sensitive criterion for intercalative binding. If the substance intercalates with DNA, it induces negative supercoiled DNA unwinding characteristic of DNA intercalators, and then DNA becomes supercoiled positively. The unwinding test of **10** using electrophoresis with pGEm7GF plasma DNA and topoisomerase I indicated that **10** might intercalate with DNA. The unwinding of circular DNA by **10** was detected at 100 μM (data not shown). Amsacrine (*N*-[4-(9-acridinylamino)-3-methoxyphenyl]methane sulfonamide) which intercalates DNA in the range of 2.5−50 μM was used as a control.

Cytotoxicity. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay developed by Mosmann[26] is a rapid and precise colorimetric tetrazolium assay for mammalian cell survival. The test compounds were dissolved in DMSO at a concentration of 1 mM and diluted with RPMI-FCS to various concentrations, respectively. Human lung adenocarcinoma cell lines

Table 2. Cytotoxicity Data on Human Lung Adenocarcinoma Cell Line (PC 14) and Human Gastric Adenocarcinoma Cell Line (MKN 45)

	IC_{50} (μM)[a]	
compd no.	PC 14	MKN 45
9	82.50	19.81
10	38.68	34.12
11	130.25	93.14
12	78.32	61.13
13	125.4	22.83
14	45.50	1.30
17	76.23	20.08
18	74	16.67
19	67.52	12.99
20	20.49	7.61
21	119.6	82.52
adriamycin	0.29	3.13
cis-platin	3.87	86.5

[a] Values are the average from three independent dose−response curves; variation was generally ±15%.

(PC 14), human gastric adenocarcinoma cell lines (MKN 45), and human colon adenocarcinoma cell lines (colon 205) were used for the cytotoxicity test in vitro. Single-cell suspensions were treated with each sample, adriamycin, and *cis*-platin in 96-well flat-bottom microplates, and the plates were incubated at 37 °C for 4 days in a 5% CO_2 incubator. The samples in RPMI-FCS were used as a blank. MTT solution was added to cells, the plates were incubated at 37 °C for an additional 4 h in a 5% CO_2 incubator, and then acid−isopropanol was added to all wells. The optical densities of plates were observed by a Microplate reader (Titertek, Multiscan) at 540 nm. Some of the compounds were colored; however, they did not show absorption at 540 nm in the highest concentration for testing. Fractional inhibition is calculated as 1 − (the ratio of optical density of sample and control); 50% inhibition concentration (IC_{50}) is defined as the compound concentration required to reduce the viability of human adenocarcinoma cells by 50%. The IC_{50} values for each compound were determined by dose−effect analysis with microcomputers (Joseph Chou and Ting-Chao Chou)[27] for comparison with adriamycin and *cis*-platin, which are summarized in Table 2. Each value is the mean of triplicate experiments. Dose−effect relationships were analyzed by the median-effect equation derived by Chou: $f_a/f_u = (D/D_m)^m$, where D is dose of samples, D_m is the median-effect dose that is required for 50% cytotoxicity (e.g., IC_{50}), f_a is the fraction affected, f_u is the fraction unaffected, and m is the Hill-type coefficient signifying the degree of the sigmoidal shape of the dose−effect curve. The correlation coefficients (R) were greater than 0.9 for all median-effect lines.

Most of the synthetic compounds (**9**−**14**, **17**−**21**) showed more potent cytotoxicity on MKN 45 than *cis*-platin. Within the series, the most cytotoxic compound against human gastric adenocarcinoma cells (MKN 45) was 2-methyl-4,9-dihydro-1-(4-bromophenyl)-1*H*-imidazo-[4,5-*g*]quinoxaline-4,9-dione (**14**) with an IC_{50} value of 1.30 μM, whereas those of adriamycin and *cis*-platin are 3.13 and 86.5 μM, respectively. The cytotoxicity of 2,3-diethyl-5,10-pyrazino[2,3-*g*]quinoxalinedione (**20**) with an IC_{50} value of 7.61 μM was comparable to that of adriamycin, and **20** is almost 10 times more active against human gastric adenocarcinoma cells (MKN 45) than *cis*-platin in vitro. The interaction energies for these two compounds are −14.648 and −14.464 kcal/mol, and these values were the lowest ones for the tested compounds. These results may support that the compounds with lower interaction energies show higher activity. Interestingly the quinoxalinediones with substituted bulky groups were more potent, which suggests that enhancement of molecular size might contribute to the cytotoxicity. However 2,3-diphenyl-5,10-pyrazino-[2,3-*g*]quinoxalinedione (**21**) bearing two phenyl groups is not potently active against MKN 45. The poor solubility of this compound seems to cause this low activity.

In the previous section, we concluded that 2-methyl-4,9-dihydro-1-methyl-1*H*-imidazo[4,5-*g*]quinoxaline-4,9-dione (**10**), as a representative of synthetic compounds, intercalates with DNA. Unfortunately this compound with an IC_{50} value of 34.12 μM, which showed almost 10-fold loss of activity compared to adriamycin but is 2.5 times more active than *cis*-platin, turned out to not be significantly cytotoxic against MKN 45. 2-Methyl-4,9-dihydro-1-(4-bromophenyl)-1*H*-imidazo[4,5-*g*]quinoxaline-4,9-dione (**14**) was considerably less active than adriamycin and *cis*-platin against human lung adenocarcinoma cell lines (PC 14). This indicates that **14** could be more selective for MKN 45 than for PC 14 and colon 205. All of the synthetic compounds did not show high cytotoxicity on human colon adenocarcinoma cells (colon 205). The pyrazinoquinoxalinediones substituted with alkyl groups at the 2 and 3 positions exhibited comparable cytotoxicity relative to the imidazoquinoxalinediones.

Conclusion

In the present report we summarize results of our survey of the cytotoxicity of synthetic compounds. This survey has revealed that imidazoquinoxalinediones bearing 4-bromophenyl substituents at the 1 position and pyrazinoquinoxalinediones bearing diethyl substituents at the 2 and 3 positions exhibited high cytotoxicity on human gastric adenocarcinoma cells (MKN 45). The IC_{50} values of 2-methyl-4,9-dihydro-1-(4-bromophenyl)-1*H*-imidazo[4,5-*g*]quinoxaline-4,9-dione (**14**) and 2,3-diethyl-5,10-pyrazino[2,3-*g*]quinoxalinedione (**20**) were 1.30 and 7.61 μM, respectively, whereas the IC_{50} values of adriamycin and *cis*-platin (used as anticancer drugs) were 3.13 and 86.5 μM, respectively. It seems that enhanced molecular area might increase the cytotoxicity. Meanwhile **14** showed a considerable loss of activity on PC 14 and colon 205. This suggests that **14** could be a very potent and selective compound against MKN 45. All synthetic compounds are coplanar annelated heterocyclic compounds with four nitrogens.

Molecular modeling study showed that 2-methyl-4,9-dihydro-1-methyl-1*H*-imidazo[4,5-*g*]quinoxaline-4,9-dione (**10**) inserted between GC/GC base pairs completely. Although modeling and experimental data indicated the possibility that **10** intercalates with DNA, it seems to not be significantly cytotoxic. The interaction energy of **10** is −11.925 kcal/mol, while the compounds **14** and **20** which are markedly cytotoxic against MKN 45 have the lowest energies of −14.648 and −14.464 kcal/mol. There is no doubt that **10** with the higher interaction energy shows lower activity than **14** and **20**. The compound **11** with the highest energy of −8.025 kcal/

mol has an IC_{50} value of 93.14 μM. It could be evident that there is a relationship between the interaction energy of an intercalation complex and cytotoxicity on MKN 45.

Experimental Section

Materials and Methods. ^{1}H and ^{13}C NMR spectra were recorded on a 300-MHz Varian Gemini NMR spectrometer. Samples were dissolved in DMSO-d_6 and $CDCl_3$. Mass spectra were obtained on Hewlett Packard 5790 (70 eV) GC−mass and GC−mass 5985B spectrometers. Elemental analyses were performed using a Perkin-Elmer model 240C elemental analyzer. IR spectra were recorded on a Perkin Elmer model 1710 spectrometer. Most reagents were purchased from Aldrich Chemical Co. 6,7-Dichloro-5,8-quinoxalinedione was prepared by Park's method.[13]

6-Azido-7-chloro-5,8-quinoxalinedione (4). Sodium azide (210 mg, 3.3 mmol) was added to a solution of 6,7-dichloro-5,8-quinoxalinedione (**3**) (500 mg, 2.2 mmol) in acetic acid (20 mL), and the reaction mixture was stirred for 30 min. The precipitate was collected by filtration and washed with water: yield, 470 mg (90.4%); mp >280 °C; IR 2129, 1675, 1695 cm^{-1}.

6-Amino-7-chloro-5,8-quinoxalinedione (5). Sodium borohydride (120 mg, 3.2 mmol) was added to a solution of azidoquinone **4** (500 mg, 2.1 mmol) in ethanol (20 mL), and the reaction mixture was stirred for 30 min. The precipitate was collected by filtration and washed with ethanol: yield, 445 mg (quantitative); mp >280 °C; ^{1}H NMR δ 7.90 (br s, 2H, NH_2, exchanges with D_2O), 8.93 (d, 2H, H-2,3); ^{13}C NMR δ 144.7, 147.1, 147.3, 147.4, 148.0, 148.3, 177.4, 177.2; IR 3444, 1710, 1598 cm^{-1}; MS *m/z* for $C_8H_4N_3O_2{}^{35}Cl$ 209; *m/z* for $C_8H_4N_3O_2{}^{37}Cl$ 211.

6-Acetamido-7-chloro-5,8-quinoxalinedione (6). A solution of aminoquinone **5** (500 mg, 2.4 mmol) and 5 drops of concentrated sulfuric acid in acetic anhydride (30 mL) was stirred in an ice bath for 1 h. After quenching with methanol slowly, the solvent was evaporated under reduced pressure. The residue was loaded on a silica gel column (Kieselgel 9385, 230−400 mesh) and eluted with *n*-hexane/ethyl acetate/ethanol (50:45:8): yield, 370 mg (61.7%); mp 230−231 °C; ^{1}H NMR δ 2.16 (s, 3H, CH_3), 9.08 (s, 2H, H-2,3), 10.28 (s, 1H, NH, exchanges with D_2O); IR 3220, 1700, 1665 cm^{-1}; MS *m/z* for $C_{10}H_6N_3O_3{}^{35}Cl$ 251, *m/z* for $C_{10}H_6N_3O_3{}^{37}Cl$ 253. Anal. ($C_{10}H_6N_3O_3Cl$) C, H, N.

6-Acetamido-7-amino-5,8-quinoxalinedione (7). Sodium azide (390 mg, 6.0 mmol) was added to a solution of acetaminoquinone **6** (500 mg, 1.99 mmol) in acetic acid (20 mL). The reaction mixture was stirred for 3 h, and the solvent was evaporated under reduced pressure. The residue was dissolved in ethanol (20 mL), and sodium borohydride (110 mg, 2.99 mmol) was added to this solution. The reaction mixture was stirred for 1 h. The precipitate was collected by filtration and washed with ethanol: yield, 250 mg (54.3%); mp 236−237 °C; ^{1}H NMR δ 2.04 (s, 3H, CH_3), 7.08 (br s, 2H, NH_2, exchanges with D_2O), 8.95 (d, 2H, H-2,3), 9.20 (br s, 1H, NH, exchanges with D_2O); IR 3286, 1695.5, 1668.5 cm^{-1}; MS *m/z* for $C_{10}H_8N_4O_3$ 232.

General Procedure for the Preparation of 2-Methylimidazo[4,5-*g*]quinoxaline-4,9-diones 9 and 14. 2-Methyl-4,9-dihydro-1*H*-imidazo[4,5-*g*]quinoxaline-4,9-dione (9). **(a)** NaOH (2 N) (1 mL) was added to a suspension of (acylamino)quinone **7** (500 mg, 2.2 mmol) in ethanol (20 mL) at 70 °C, and the suspension was refluxed for 30 min. The reaction mixture was poured into water (5 mL) with 2 N HCl (1 mL). After cooling, the precipitate was collected by filtration, loaded on a silica gel column (Kieselgel 9385, 230−400 mesh), and eluted with chloroform/acetone (9:1).

(b) The HCl gas was passed through a suspension of (acylamino)quinone **7** (500 mg, 2.2 mmol) in methanol (20 mL) for 1 h, and the suspension was refluxed for 1.5 h. The reaction mixture was neutralized with sodium bicarbonate, and the solvent was evaporated under reduced pressure. The residue was crystallized from ethanol: yield, 370 mg (80.4%); mp >280 °C; ^{1}H NMR δ 2.29 (s, 3H, CH_3), 8.80 (s, 1H, H-6,7); ^{13}C NMR δ 174.7, 162.5, 146.9, 146.6, 146.2, 146.1, 145.9, 17.5; IR 3480, 1683.9 cm^{-1}; MS *m/z* for $C_{10}H_6N_4O_2$ 214. Anal. ($C_{10}H_6N_4O_2 \cdot H_2O$) C, H, N.

2-Methyl-4,9-dihydro-1-(4-bromophenyl)-1*H*-imidazo[4,5-*g*]quinoxaline-4,9-dione (14): yield, 250 mg (86.2%); mp 279−281 °C; ^{1}H NMR δ 2.36 (s, 3H, CH_3), 7.57 (d, 2H, J = 7.7 Hz, H-3′,5′), 7.85 (d, 2H, J = 7.7 Hz, H-2′,6′), 9.04 (d, 2H, H-6,7); ^{13}C NMR δ 176.06, 171.7, 153.8, 147.7, 147.5, 134.2, 133.9, 132.8, 132.5, 132.1, 129.2, 129.1, 122.9, 13.6; IR 1680 cm^{-1}; MS *m/z* for $C_{16}H_9N_4O_2{}^{79}Br$ 368, *m/z* for $C_{16}H_9N_4O_2{}^{81}Br$ 370.

General Procedure for the Preparation of 2-Methyl-1-substituted-imidazo[4,5-*g*]quinoxaline-4,9-diones 10−13 and 6-Acetamdo-7-[(4-bromophenyl)amino]-5,8-quinoxalinedione (8). 2-Methyl-4,9-dihydro-1-methyl-1*H*-imidazo[4,5-*g*]quinoxaline-4,9-dione (10). Methylamine (40% in water, 0.19 mL) was added to a suspension of acetaminoquinone **6** (300 mg, 1.3 mmol) in ethanol (20 mL) at 70 °C, and the reaction mixture was refluxed for 30 min. The solvent was evaporated under reduced pressure, and the residue was loaded on a silica gel column (Kieselgel 9385, 230−400 mesh) and eluted with *n*-hexane/ethyl acetate/ethanol (50:45:8): yield, 210 mg (77.8%); mp 278−280 °C; ^{1}H NMR δ 2.52 (s, 3H, $COCH_3$), 3.97 (s, 3H, NCH_3), 9.03 (s, 2H, H-6,7); IR 1630 cm^{-1}. Anal. ($C_{11}H_8N_4O_2$) C, H, N.

2-Methyl-4,9-dihydro-1-ethyl-1*H*-imidazo[4,5-*g*]quinoxaline-4,9-dione (11): yield, 220 mg (75.9%); mp 278−280 °C; ^{1}H NMR δ 1.36 (t, 3H, J = 7.14 Hz, CH_3), 2.56 (s, 3H, CH_3), 4.43 (q, 2H, J = 7.14 Hz, CH_2), 9.02 (s, 2H, H-6,7); IR 1680 cm^{-1}. Anal. ($C_{12}H_{10}N_4O_2$) C, H, N.

2-Methyl-4,9-dihydro-1-isopropyl-1*H*-imidazo[4,5-*g*]quinoxaline-4,9-dione (12): yield, 230 mg (74.2%); mp >280 °C; ^{1}H NMR δ 1.58 (d, 6H, dime.), 2.63 (s, 3H, CH_3), 5.08 (m, 1H, CH), 9.03 (d, 2H, H-6,7); IR 1670 cm^{-1}; MS *m/z* for $C_{13}H_{12}N_4O_2$ 256. Anal. ($C_{13}H_{12}N_4O_2 \cdot {}^3/_2H_2O$) C, H, N.

2-Methyl-4,9-dihydro-1-phenyl-1*H*-imidazo[4,5-*g*]quinoxaline-4,9-dione (13): yield, 230 mg (65.7%); mp >300 °C; ^{1}H NMR δ 2.33 (s, 3H, CH_3), 7.61−7.55 (m, 5H, ArH), 9.02 (d, 2H, H-6,7); IR 1643.5 cm^{-1}; MS *m/z* for $C_{16}H_{10}N_4O_2$ 290. Anal. ($C_{16}H_{10}N_4O_2 \cdot H_2O$) C, H; N: calcd, 19.31; found, 20.01.

6-Acetamdo-7-[(4-bromophenyl)amino]-5,8-quinoxalinedione (8): yield, 420 mg (91.3%); mp 270−272 °C; ^{1}H NMR δ 1.42 (s, 3H, CH_3), 6.91 (d, 2H, J = 8.7 Hz, H-3′,5′), 7.40 (d, 2H, J = 8.7 Hz, H-2′,6′), 9.00 (d, 1H, J = 9.89 Hz, H-2), 9.01 (d, 1H, J = 9.89 Hz, H-3), 9.32 (s, 1H, NH, exchanges with D_2O), 9.41 (s, 1H, NH (4-bromoaniline), exchanges with D_2O); MS *m/z* for $C_{16}H_{11}N_4O_3{}^{79}Br$ 386, *m/z* for $C_{16}H_{11}N_4O_3{}^{81}Br$ 388.

6,7-Diazido-5,8-quinoxalinedione (15). Sodium azide (430 mg, 10 mmol) was added to a solution of 6,7-dichloro-5,8-quinoxalinedione (**3**) (500 mg, 2.19 mmol) in DMF (20 mL), and the solution was stirred at room temperature for 30 min. The precipitate was collected by filtration and washed with water: yield, 500 mg (94.3%); mp >280 °C; IR 2080, 1660 cm^{-1}.

6,7-Diamino-5,8-quinoxalinedione (16). (a) Sodium borohydride (200 mg, 6.2 mmol) was added to a solution of diazidoquinone **15** (500 mg, 2.07 mmol) in ethanol (20 mL), and the solution was stirred for 30 min at 70−80 °C. The precipitate was collected by filtration and washed with aqueous ethanol.

(b) Potassium hydroxide (100 mg, 3.2 mmol) was added to a suspension of (acylamino)quinone **7** (500 mg, 2.2 mmol) in ethanol (20 mL), and the reaction mixture was refluxed for 1 h. After cooling, the precipitate was collected by filtration and recrystallized from ethanol: yield, 290 mg (74.4%); mp >280 °C; ^{1}H NMR δ 5.84 (s, 4H, each NH_2, exchanges with D_2O), 8.70 (s, 2H, H-2,3); ^{13}C NMR δ 144.94, 146.08, 147.51, 195.27; IR 3460, 3380, 1630 cm^{-1}; MS *m/z* for $C_8H_6N_4O_2$ 190.

5,10-Pyrazino[2,3-*g*]quinoxalinedione (17). Sodium borohydride (200 mg, 6 mmol) was added to a solution of diazidoquinone **15** (500 mg, 2 mmol) in ethanol (20 mL), and the reaction mixture was stirred at 40 °C for 1 h. The solvent

was evaporated under reduced pressure, and the residue was suspended in water (50 mL). Glyoxal (40% in water, 2 mL) was added to this suspension at 90 °C, and the reaction mixture was stirred for 1 h. After cooling, the precipitate was collected by filtration and recrystallized from ethanol: yield, 210 mg (47.7%); mp >280 °C; ^{1}H NMR δ 9.18 (s, H-2, 3, 6, and 7); IR 1732.2 cm^{-1}; MS *m*/*z* for $C_{10}H_4N_4O_2$ 212.

General Procedure A for the Preparation of 2,3-Disubstituted-5,10-pyrazino[2,3-*g*]quinoxalinediones 18−20. 2,3-Dimethyl-5,10-pyrazino[2,3-*g*]quinoxalinedione (18). Sodium borohydride (200 mg, 6 mmol) was added to a solution of diazidoquinone **15** (500 mg, 2 mmol) in ethanol (20 mL), and the reaction mixture was stirred at 30−40 °C for 1 h. After cooling, the precipitate was filtered and washed with 50% aqueous ethanol (50 mL). The ethanol was evaporated under reduced pressure, and the residue was diluted with acetic acid to 50 mL. 2,3-Butanedione (0.5 mL, 6 mmol) was added to this solution, and the reaction mixture was stirred at room temperature for 30 min. Water was added to this mixture, the mixture was extracted with chloroform, and the organic layer was dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure, and the residue was loaded on a silica gel column (Kieselgel 9385, 230−400 mesh) and eluted with *n*-hexane/ethyl acetate/ethanol (50:45:8). After evaporation of solvent from the pooled fractions the precipitate was recrystallized from *n*-hexane and ethyl acetate.

General Procedure B. Sodium borohydride (200 mg, 6 mmol) was added to a solution of diazidoquinone **15** (500 mg, 2 mmol) in ethanol (20 mL), and the reaction mixture was stirred at 40 °C for 1 h. After cooling, the precipitate was filtered and washed with 50% aqueous ethanol (50 mL). The ethanol was evaporated under reduced pressure, and the residue was diluted with acetic acid to 50 mL. 1,4-Dibromo-2,3-butanedione (1.5 g, 6 mmol) was added to this solution, and the reaction mixture was refluxed for 2.5 h. Water was added to this mixture, the mixture was extracted with chloroform, and the organic layer was dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure, and the residue was crystallized from methanol: yield, 450 mg (90.9%); mp 245−246 °C; ^{1}H NMR δ 9.18 (s, 2H, H-7,8), 2.89 (s, 6H, dime.); ^{13}C NMR δ 179.5, 160.3, 149.7, 144.7, 142.3, 23.2; IR 1701.3 cm^{-1}; MS *m*/*z* for $C_{12}H_8N_4O_2$ 240. Anal. ($C_{12}H_8N_4O_2 \cdot {}^1/_2H_2O$) C, H, N.

2-Methyl-3-ethyl-5,10-pyrazino[2,3-*g*]quinoxalinedione (19). 19 was prepared using general procedure A: yield, 420 mg (80.8%); mp 232−233 °C; ^{1}H NMR δ 9.17 (s, 2H, H-7,8), 3.16 (q, 2H, J = 7.4 Hz, CH_2 of C-3), 2.9 (s, 3H, CH_3), 1.45 (t, 3H, J = 7.4 Hz, CH_3 of C-3); ^{13}C NMR δ 179.58, 179.46, 164.44, 159.79, 149.65, 144.71, 144.62, 142.43, 142.04, 32.9, 29.2, 11.92; IR 1703.3 cm^{-1}; MS *m*/*z* for $C_{13}H_{10}N_4O_2$ 254. Anal. ($C_{13}H_{10}N_4O_2 \cdot {}^1/_2H_2O$) C, H, N.

2,3-Diethyl-5,10-pyrazino[2,3-*g*]quinoxalinedione (20). 20 was prepared using general procedure A: yield, 460 mg (83.6%); mp 256−257 °C; ^{1}H NMR δ 9.17 (s, 2H, H-7,8), 3.19 (q, 4H, J = 7.4 Hz, each CH_2), 1.47 (t, 6H, J = 7.4 Hz, each CH_3); ^{13}C NMR δ 179.5, 163.9, 149.8, 144.6, 142.2, 29.7, 28.6, 12.6; IR 1703.3 cm^{-1}. Anal. ($C_{14}H_{12}N_4O_2 \cdot {}^3/_5H_2O$) C, H, N.

2,3-Diphenyl-5,10-pyrazino[2,3-*g*]quinoxalinedione (21). Sodium borohydride (200 mg, 6 mmol) was added to a solution of diazidoquinone **15** (500 mg, 2 mmol) in ethanol (20 mL), and the reaction mixture was stirred at 30−40 °C for 1 h. Benzil (700 mg, 6 mmol) and 5 drops of concentrated sulfuric acid were added to this solution, and the reaction mixture was refluxed for 1 h. The solvent was evaporated under reduced pressure, and the residue was loaded on a silica gel column (Kieselgel 9385, 230−400 mesh) and eluted with *n*-hexane/ethyl acetate/ethanol (50:45:8). After evaporation of solvent from the pooled fractions, the precipitate was recrystallized from DMSO: yield, 490 mg (65.3%); mp 263−265 °C; ^{1}H NMR δ 9.20 (d, 2H, H-7,8), 8.38 (m, 4H, each H-2′,6′), 7.7 (m, 6H, each H-3′,4′,5′); ^{13}C NMR δ 175.31, 155.21, 151.22, 147.49, 137.53, 136.62, 132.26, 131.33, 125.65, 117.54, 114.5; IR 1701.3 cm^{-1}.

Acknowledgment. The authors wish to thank Il Dong Pharm. Co. for its donation of the Chair Fund to KIST, Dr. Yun-Sil Lee (Laboratory of Radiation Effect, Korea Cancer Hospital) for assistance in obtaining and interpreting the cytotoxicity data, and Dr. Seog K. Kim (Department of Chemistry, Yeungnam University) for assistance in obtaining and interpreting the UV spectrum. This work was partially supported by the Korea Science Engineering Foundation (KOSEF).

References

(1) Rao, K. V.; Cullen, W. P. Streptonigrin, An antitumor substance, I. Isolation and characterization. *Antibiot. Ann.* **1959**, 950−953.
(2) Ebert, P. S.; Chirigos, M. A.; Ellsworth, P. A. Differential response of Friend leukemia virus and lactate dehydrogenase virus to chemotherapy and in vitro neutralization. *Cancer Res* **1968**, *28*, 363−367.
(3) Kaung, D. T.; Whittington, R. M.; Spencer, H. H.; Patno, M. E. Comparison of chlorambucil and streptonigrin (NSC-45383) in the treatment of malignant lymphomas. *Cancer* **1969**, *23*, 597−600.
(4) Chirigos, M. A.; Pearson, J. W.; Papas, T. S.; Woods, W. A.; Wood, H. B.; Spahn, G. Effect of streptonigrin (NSC-45383) and analogs on oncornavirus replication and DNA polymerase activity. *Cancer Chemother. Rep., Part 1* **1973**, *57*, 305−309.
(5) Inouye, Y.; Take, Y.; Oogose, K.; Kubo, A.; Nakamura, S. The quinolinequinone as the minimum entity for reverse transcriptase-inhibitory activity of streptonigrin. *J. Antibiot.* **1987**, *40*, 105−107.
(6) Shaikh, I. A.; Johnson, F.; Grollman, A. P. Streptonigrin. 1. Structure-activity relationships among simple bicyclic analogues. Rate dependence of DNA degradation on quinone reduction potential. *J. Med. Chem.* **1986**, *29*, 1329−1340.
(7) Rao, K. V.; Rock, C. P. Streptonigrin and related compounds. 6. Synthesis and activity of some quinoxaline analogues. *J. Heterocycl. Chem.* **1996**, *33*, 447−458.
(8) Rao, K. V. Quinone natural products: streptonigrin (NSC-45383) and lapachol (NSC-11905) structure-activity relationships. *Cancer Chemother. Rep., Part 2* **1974**, *4*, 11−17.
(9) Lin, A. J.; Lillis, B. J.; Sartorelli, A. C. Potential bioreductive alkylating agents. 5. Antineoplastic activity of quinoline-5,8-diones, naphthazarins, and naphthoquinones. *J. Med. Chem.* **1975**, *18*, 917−921.
(10) Lown, J. W.; Joshus, A. V.; Lee, J. S. Molecular mechanisms of binding and single strand scisson of DNA by the antitumor antibiotics saframycins A and C. *Biochemistry* **1982**, *21*, 419−428.
(11) Yamashita, Y.; Tsubata, Y.; Suzuki, T.; Miyashi, T.; Mukai, T.; Tanaka, Benazo[g][1,2,5]thiadiazolo[3,4-b]quinoxaline-5,10-dione and its selenium analog. An unusual type of quinones. *Chem. Lett.* **1990**, *1990*, 445−448.
(12) Renault, S. G.; Renault, J.; Baron, M.; Servolles, P.; Paoletti, C.; Cros, S. Heterocyclic quinones. VI: synthesis and antitumoral effects of 5,10-benzo[g]quinoxalinediones and aza-analogues. *Eur. J. Med. Chem.-Chim. Ther.* **1985**, *20* (2), 144−148.
(13) Han, G. H.; Shin, K. J.; Kim, D. C.; Yoo, K. H.; Kim, D. J.; Park, S. W. A new synthetic route to 6,7-dichloro-5,8-quinoxalinedione and synthesis of its derivatives. *Heterocycles* **1996**, *43*, 2495−2502.
(14) Saenger, W. *Principles of Nucleic Acid Structure*; Springer: New York, 1983.
(15) Hoover, J. R. E.; Day, A. R. Preparation of some imidazole derivatives of 1,4-naphthoquinone. *J. Am. Chem. Soc.* **1954**, *76*, 4148−4152.
(16) Fieser, L. F.; Martin, E. L. A comparison of heterocyclic systems with benzene. VII. Isologs of anthraquinone containing one and two triazole rings. *J. Am. Chem. Soc.* **1935**, *57*, 1844−1849.
(17) Winkelmann, E. 2,3,5,6-Tetraamino-1,4-benzoquinone. Preparation, properties, and reactions. *Tetrahedron* **1969**, *25*, 2427−2454.
(18) Nakazumi, H.; Agawa, T.; Kitao, T. Synthesis and absorption spectra of 1,4-diazaanthraquinone derivatives. *Bull. Chem. Soc. Jpn.* **1979**, *52* (8), 2445−2446.
(19) Suschitzky, H.; Kramen, W.; Neidlein, R.; Uhl, H. 2H-Benzimidazoles (isobenzimidazoles). Part 2. Synthesis and reacions of 5-azido-2H-benzimidazoles-2-spirocyclohexane. *J. Chem. Soc. Perkin Trans 1* **1988**, 983−989.
(20) Efimova, G. A.; Efros, L. S. Heterocyclic derivatives based on substituted 1,4-naphthoquinones. II. Synthesis and properties of 2,3-disubstituted-benzoquinoxalin-5,10-diones. *Zh. Organ. Khim. (Russ.)* **1966**, *2*, 531−536.

(21) Kitahara, Y.; Nakahara, S.; Tanaka, Y.; Kubo, A. Synthesis of 5,8-quinoxalinediones and 5,8-quinazolinediones. *Heterocycles* **1992**, *34* (8), 1623−1630.
(22) Berman, H. M.; Young, P. R. The interaction of intercalating drugs with nucleic acids. *Annu. Rev. Biophys. Bioeng.* **1981**, *10*, 87−114.
(23) Pindur, U.; Haber, M.; Sattler, K. Antitumor active drugs as intercalators of deoxyribonucleic acid. *J. Chem. Educa.* **1993**, *70* (4), 263−272.
(24) Jain, S. C.; Bhandary, K. K.; Sobell, H. M. Visualization of drug-nuclei acid interactions at atomic resolution. *J. Mol. Biol.* **1979**, *135*, 813−840.
(25) Keller, W. Determination of the number of superhelical turns in simian virus 40 DNA by gel electrophoresis. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72* (12), 4876−4880.
(26) Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **1983**, *65*, 55−63.
(27) Chou, J.; Chou, T. C. *Dose-effect analysis with microcomputers: quantiation of* ED_{50}, LD_{50}, *synergism, antagonism, low-dose risk, receptor-ligand binding and enzyme kinetics*; manual and software; Biosoft: Cambridge, England, 1987.

JM970695N